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Detection of Human Herpesvirus 7 (HHV-7) DNA in Breast Milk by Polymerase Chain Reaction and Prevalence of HHV-7 Antibody in Breast-Fed and Bottle-Fed Children

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Twenty-nine breast milk mononuclear cell samples were analyzed for human herpesvirus 7 (HHV-7) DNA, human herpesvirus 6 (HHV-6) DNA, and human cytomegalovirus (HCMV) DNA by polymerase chain reaction (PCR). In addition, peripheral blood mononuclear cell samples from 13 puerperants were analyzed for HHV-7 DNA by PCR, and seropositivity of HHV-7 was also analyzed in breast-fed and bottle-fed children. HHV-7 DNA was detected in 3 of 29 breast milk samples. HCMV DNA was also detected in 3 of 29 breast milk samples, but HHV-6 DNA was not detected. HHV-7 DNA was detected in 11 of 13 samples of peripheral blood mononuclear cells. Though the seropositivity rate for HHV-7 in breast-fed children was slightly higher than that in bottle-fed children at 18 and 24 months old, the difference was not statistically significant. From these results, we speculate that breast-feeding may be one of the transmission routes of HHV-7, although this is not the main route. ***J. Med. Virol. 56:275–279, 1998.***

KEY WORDS: breast-feeding; HHV-7 transmission; mother-to-child; breast milk mononuclear cells; PCR; seroepidemiology

INTRODUCTION

Human herpesvirus 7 (HHV-7) was first isolated from $CD4^+$ T-cells activated from the peripheral blood mononuclear cells (PBMNC) of a healthy individual in 1990 [Frenkel et al., 1990]. Subsequently, HHV-7 has been isolated from the saliva of healthy adults [Wyatt and Frenkel, 1992] and the peripheral blood of a patient with chronic fatigue syndrome [Berneman et al., 1992a]. However, no association between HHV-7 infection and chronic fatigue syndrome was found by seroepidemiologic studies [Berneman et al., 1992b]. Although the clinical symptoms caused by HHV-7 have not been completely defined, we recently reported that HHV-7 is one of the causative agents of exanthem subitum [Tanaka et al., 1994]. Seroepidemiologic studies have indicated that primary infection with HHV-7 occurs during childhood [Wyatt et al., 1991] similarly to human herpesvirus 6 (HHV-6), and oral transmission is regarded as the main route of HHV-7 infection. In this report, we describe detection of HHV-7 DNA in breast milk mononuclear cells (MNC) and frequent detection of HHV-7 DNA in PBMNC from puerperants by polymerase chain reaction (PCR). Though the seropositivity rate for HHV-7 in breast-fed children was slightly higher than that in bottle-fed children at 18 and 24 months old, the difference was not statistically significant. From these results, we speculate that breast-feeding may be one of the transmission routes of HHV-7, although this is not the main route.

MATERIALS AND METHODS

Samples

Ten to 20 ml of breast milk was obtained from 29 puerperants at various times (from 2 to 24 days) after delivery at Aizenbashi Hospital. Twenty-four subjects allowed us to collect peripheral blood at the same time. One hundred and forty-one serum samples were also

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collected at Osaka University Hospital from 64 children at 12, 18, or 24 months old. Informed consent for blood and/or breast milk sampling was obtained from all of the puerperants or the children's parents.

Antibody Detection

The indirect immunofluorescence test for serum antibody to HHV-7 was performed as described previously [Okuno et al., 1989; Tanaka et al., 1994] with some modifications. Briefly, SUP-T1 cells, an immature T-cell line [Berneman et al., 1992b], were infected with HHV-7 (7-KHR strain [Tanaka et al., 1994]). Approximately 3 or 4 days after infection, when 40% to 50% of cells showed cytopathic effects, they were mounted on 24-well glass slides and fixed in acetone at –20°C for 15 min. Uninfected cells on 24-well glass slides were also prepared in the same fashion. Serially twofold diluted sera were placed on the slides and incubated for 1 hr at 37°C. The slides were then washed twice with phosphate-buffered saline (PBS), treated with anti human IgG antibody labeled with fluorescein isothiocyanate (Dako, Copenhagen, Denmark), and incubated for 1 hr. After washing twice with PBS, the slides were observed under a fluorescence microscope. Titers of the antibodies were expressed as the highest serum dilution yielding detectable immunofluorescence. Serological cross reactivity with HHV-6 was excluded because there were patients who were positive for HHV-7 only or HHV-6 only, as reported previously [Tanaka et al., 1994].

Statistical Analysis

The difference between the HHV-7 seroprevalence of breast-fed and bottle-fed children was statistically analyzed by the χ^2 test or the Fisher's exact test using StatView software, version J-4.02 (Abacus Concepts, Berkeley, CA).

Preparation of Mononuclear Cells and Supernatant From Breast Milk

Each breast milk sample was centrifuged at 2,000 rpm for 10 min and the supernatant was separated. The supernatant was frozen at –80°C until use. After the sedimented cells were washed once with PBS and resuspended in 3 ml of PBS, mononuclear cells resuspended in PBS were separated with Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden) gradient centrifugation. Aliquots of 2×10^6 cells were washed with PBS again and frozen at –80°C until use for PCR.

Preparation of Mononuclear Cells From Peripheral Blood

PBMNC from 13 of 24 puerperants were separated with Ficoll-Paque gradient centrifugation. They were washed with PBS and aliquots of 2×10^6 PB MNC were frozen at –80°C.

PCR

For PCR analysis, aliquots of 2×10^6 cells were suspended in 200 μl of K buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 3 mM $MgCl_2$, 0.45% NP40, 0.45% Tween 20, and 100 μg/ml of proteinase K) and incubated at 56°C for 3 hr. After inactivation of proteinase K at 95°C for 10 min, 10 μl of the solution was subjected to PCR analysis as template DNA, which was equivalent to that derived from 1×10^5 cells. From supernatant of breast milk, genomic DNA was prepared by sodium iodide method using DNA Extractor kit (Wako pure chemical industries, Osaka, Japan) according to the manufacturer's protocol. Prepared DNA from 100 μl of supernatant was subjected to PCR.

The PCR methods for HHV-7 and HCMV were previously described [Tanaka-Taya et al., 1996; Miyoshi et al., 1998]. The methods for HHV-6 were also described previously [Yalcin et al., 1994; Yamamoto et al., 1994] and performed with some modifications as follows. Its conditions consisted of 30 cycles of a denaturation step of 1 min at 94°C, an annealing step of 2 min at 62°C, and an elongation step of 3 min, 4 min, and 5 min each for 10 cycles at 72°C. Amplified products were visualized on 2% agarose gels stained with ethidium bromide.

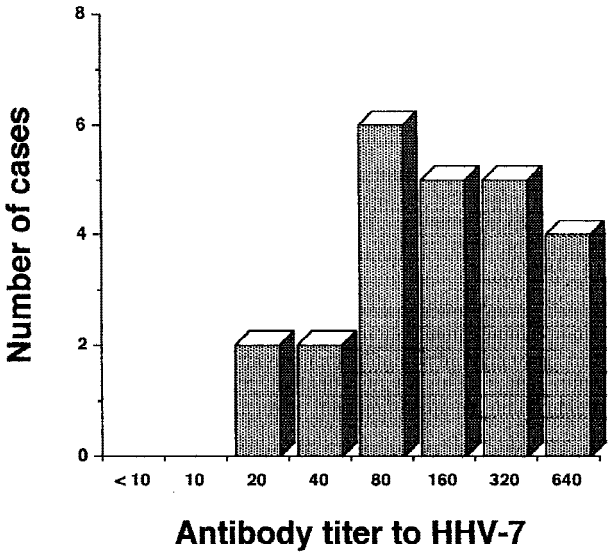

Fig. 1. HHV-7 antibody titers in puerperants. Triplicate experiments were performed in each sample and the results were read by two observers. The titer that is equal to or is more than 1:10 was considered positive. No background was obtained with negative sera in this assay.

Detection of Amplified Product

The results of PCR amplification were confirmed by hybridization with alkaline phosphatase-conjugated oligonucleotide probes. The methods for detection of HHV-7, HHV-6, and HCMV DNA were previously described, and the sensitivity of these assays for HHV-7, HHV-6, and HCMV were 10 to 100, 1 to 10, and 1 to 10 genome equivalents, respectively [Tanaka-Taya et al., 1996; Miyoshi et al., 1998].

RESULTS

All 24 puerperants' serum samples were positive for HHV-7 antibody (Fig. 1). Among 141 serum samples

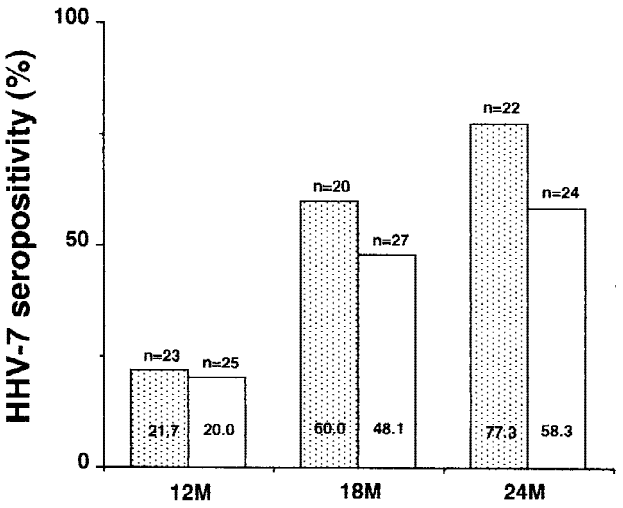

Fig. 2. Prevalence of HHV-7 antibody in breast-fed and bottle-fed children. Seropositivity was evaluated as described in Figure 1. Shaded bars represent breast-fed; blank bars, bottle-fed.

from 64 children, 65 were from 30 breast-fed children (23 samples at 12 months old, 20 samples at 18 months old, and 22 samples at 24 months old) and 76 were from 34 bottle-fed children (25 samples at 12 months old, 27 samples at 18 months old, and 24 samples at 24 months old). The positive rates of HHV-7 antibody of breast-fed children were 5/23 (21.7%) at 12 months old, 12/20 (60.0%) at 18 months old, and 17/22 (77.3%) at 24 months old. Those of bottle-fed children were 5/25 (20.0%) at 12 months old, 13/27 (48.1%) at 18 months old, and 14/24 (58.3%) at 24 months old (Fig. 2). However, no positive staining was observed when uninfected cells were used. By the χ^2 test or the Fisher's exact test, there were no significant differences between breast-fed and bottle-fed children at 12, 18, and 24 months old ($P > 0.9999$, = 0.1452, and = 0.1711, respectively).

HHV-7 DNA was detected in 3 (10.3%) of the 29 breast milk MNC samples. HCMV DNA was also detected in 3 (10.3%) breast milk MNC samples, but HHV-6 DNA was not detected (Table I). There was no breast milk MNC sample in which both HHV-7 DNA and HCMV DNA were detected. One puerperant, in whose breast milk MNC HHV-7 DNA was detected, was also analyzed for HHV-7 DNA in supernatant of centrifuged breast milk, but it was not detected. HHV-7 DNA was detected in 11 of 13 samples of puerperants' PBMNC. Of three puerperants in whose breast milk MNC HHV-7 DNA was detected, two allowed us to collect peripheral blood, and HHV-7 antibody titers of their sera were 1:80 and 1:640. But HHV-7 DNA was not detected in one of them from peripheral blood and we did not analyze for HHV-7 DNA in the other.

TABLE I. Detection of Virus DNA in 29 Breast Milk MNC Samples

	HHV-7	HHV-6	HCMV
Positive	3 (10.3%)	0 (0.0%)	3 (10.3%)
Negative	26 (89.7%)	29 (100%)	26 (89.7%)

DISCUSSION

To investigate the possibility of mother-to-child HHV-7 transmission via breast-feeding, we first focused in this study on the detection of HHV-7 DNA in breast milk MNC by PCR comparing to other β-herpesviruses, HHV-6 and HCMV, which were reported not to be transmitted and to be transmitted via breast milk, respectively.

The modes of transmission of HCMV have been well defined. In infants and children, transmission involves intrauterine infection from mothers infected with the virus (mostly primary infection) and perinatal infection, either by exposure to vaginal secretions during delivery in the mother's genital tract or by breast feeding. The prolonged period of intermittent shedding of virus in body fluids after infection may be a major source of HCMV transmission in children [Shen et al., 1992].

In the early 1970s, Hayes et al. [1972] reported that HCMV was isolated from maternal breast milk of 17 of 63 seropositive women sampled from 2 days to 10 weeks after delivery, and then concluded that breast milk must rank with cervical secretions as a potentially important source of HCMV infection in infants. HCMV has been isolated from breast milk of 13–42% of mothers and 58–69% of infected milk led to HCMV infection of infants [Stagno et al., 1980; Dworsky et al., 1983]. Minamishima et al. [1994] compared the prevalence of IgG antibody against HCMV between breast-fed and bottle-fed children, and they suggested that about 40% of the breast-fed children acquire HCMV via breast milk. In this study, we detected HCMV DNA in 10.3% of breast milk MNC samples by PCR and this result was consistent with previous reports above.

The modes of transmission of HHV-6 and HHV-7 are not yet fully clear, but saliva is the speculative source of transmission of both viruses because HHV-6 was detected in saliva by PCR [Gopal et al., 1990; Jarret et al., 1990; Kido et al., 1990] and HHV-7 was isolated at the high frequency from the saliva of healthy adults [Wyatt and Frenkel, 1992; Black et al., 1993; Hidaka et al., 1993; Yoshikawa et al., 1993]. Moreover, we recently reported that the mode of transmission of HHV-6 appears to be horizontal, mainly from mother to child during infancy [Mukai et al., 1994], and sometimes from other babies in closed institutions such as orphanages, as we found an outbreak of exanthem subitum in an orphanage and the DNA of isolated viruses showed identical restriction enzyme patterns, suggesting that the same virus was transmitted to other children [Okuno et al., 1991].

On the other hand, breast milk is not regarded as a common vehicle for the transmission of HHV-6 because HHV-6 DNA was not detected in human breast milk samples in either a previous study [Dunne and Jevon, 1993] or this one. To our knowledge, there have been

few studies in which maternal breast milk was examined for HHV-7. In this study, HHV-7 DNA was detected in 3 of 29 human breast milk MNC samples by PCR. As breast milk was collected only once from each puerperant from 2 to 29 days after delivery, the incidence of mothers in whose breast milk MNC HHV-7 exists during lactation is not clear. However, if the presence of HHV-7 in breast milk was analyzed throughout the period of lactation, it is expected that the positivity rate of HHV-7 in breast milk MNC of mothers would be higher than that reported here. From the detection of HHV-7 DNA in breast milk MNC, cell-associated HHV-7 existence in breast milk is revealed. The results of analyses for HHV-6 and HCMV DNA in breast milk MNC using same method in this study were consistent with previous studies of HHV-6 and HCMV mother-to-child infections via breast milk. Taking together these results, it is suspected that cell-associated HHV-7 in breast milk can infect children via breast-feeding, such as human T-cell leukemia virus type I, but the role of extracellular virus in infection is not clear because only one supernatant sample was analyzed in this study.

If breast-feeding is the main route of transmission of HHV-7, seropositivity for HHV-7 in breast-fed children should have been higher than that in bottle-fed children. In this study, however, the difference of seropositivity rate for HHV-7 between breast-fed and bottle-fed children at any age was not statistically significant, though the rate in breast-fed children was slightly higher than that in bottle-fed children at 18 and 24 months old. This difference at 18 and 24 months old may become significant statistically in future studies of more children, but there should have been a difference even at 12 months old or younger if HHV-7 infects children mainly via breast-feeding.

In this study, we also frequently detected HHV-7 DNA in PBMNC from puerperants, and HHV-7 is a virus that was first isolated from the PBMNC of a healthy individual [Frenkel et al., 1990]. From these results, we speculate that HHV-7, which exists commonly in adult PBMNC, exists also in breast milk MNC in some or most mothers and infects infants via breast-feeding, although this is not the main route of mother-to-child transmission of HHV-7. If HHV-6 is not transmitted and HHV-7 is transmitted by breast milk, HHV-7 infection should precede HHV-6 infection. Some previous reports, however, suggested that infection with HHV-7 occurs somewhat later than HHV-6 infection [Wyatt et al., 1991], which seems to occur before 24 months of age [Yamanishi et al., 1988; Okuno et al., 1989]. The reason why HHV-7 infection tends to follow HHV-6 infection is not clear, but it seems not to be caused by virus existence in maternal breast milk. We speculate that some factor of children, for example immaturity of HHV-7 receptor, is associated with this phenomenon.

In summary, our results indicated that breast-feeding may be one of the routes, although not the main one, of transmission of HHV-7.

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